Journal of Chemical Ecology, Vol. 13, No. 7, 1987

IDENTIFICATION OF FEMALE-PRODUCED SEX PHEROMONE FROM BANDED CUCUMBER BEETLE, *Diabrotica balteata* LECONTE (COLEOPTERA: CHRYSOMELIDAE)[1]

T. CHUMAN,[2] P.L. GUSS,[3] R.E. DOOLITTLE, J.R. McLAUGHLIN, J.L. KRYSAN,[4] J.M. SCHALK,[5] and J.H. TUMLINSON

Insect Attractants, Behavior, and Basic Biology Research Laboratory
Agricultural Research Service, U.S. Department of Agriculture
Gainesville, Florida 32604

(Received July 3, 1986; accepted September 29, 1986)

Abstract—A sex pheromone produced by female banded cucumber beetle adults, *Diabrotica balteata* LeConte, was isolated from volatiles trapped on Porapak Q and identified as 6,12-dimethylpentadecan-2-one. The structure was elucidated by spectroscopic analyses and confirmed by synthesis. The synthesized racemic compound was equal to the purified natural pheromone in eliciting responses by banded cucumber beetle males to field traps. A dose–response characteristic was demonstrated for the racemic material formulated on filter paper or rubber septa and placed in field traps. The absolute configuration at the C-6 and C-12 positions was not established.

Key Words—Sex pheromone, 6,12-dimethylpentadecan-2-one, ketone, banded cucumber beetle, *Diabrotica balteata*, Chrysomelidae, Coleoptera, attractant.

INTRODUCTION

The banded cucumber beetle (BCB), *Diabrotica balteata* LeConte, an economic pest of vegetable and field crops, particularly sweet potato and seedling

[1] This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or the recommendation for its use by USDA.
[2] Visiting scientist, Japan Tobacco Inc., Yokohama, Kanagama 227, Japan.
[3] This research was initiated by Dr. P.L. Guss, deceased, Research Chemist, Northern Grain Insects Research Laboratory, ARS, USDA, Brookings, South Dakota. Dr. Guss conducted the preliminary purifications and bioassays that led to the isolation and identification of this pheromone.
[4] Yakima Agricultural Research Laboratory, ARS, USDA, Yakima, Washington 98702.
[5] Vegetable Insects Research Laboratory, ARS, USDA, Charleston, South Carolina 29407.

0098-0331/87/0700-1601$05.00/0

cucurbits (Pitre and Kantack, 1962; Saba, 1970; Teng et al., 1984; Young, 1961), occurs from southern United States to Colombia, Venezuela, and Cuba (Krysan, 1986).

Since the use of broad-spectrum insecticides to control BCB often causes outbreaks of secondary pests, a number of studies on host performance of BCB adults have been conducted in efforts to develop noninsecticidal methods to manage this pest (Melhus et al., 1954; Cuthbert and Jones, 1972; Overman and MacCarter, 1972; Risch, 1976). The presence of a BCB female-produced sex pheromone, which should be useful in monitoring and possibly in controlling this species, was reported by Cuthbert and Reid (1964). Later Schwarz et al. (1971) reported that the BCB pheromone molecule included *n*-dodecenyl, ethylene epoxy, and methyl ketone moieties, but they were unable to complete the structure elucidation.

We report here the isolation, identification, and synthesis of a sex pheromone, collected from BCB female-produced volatiles, that attracts males of the species in the field.

METHODS AND MATERIALS

Pheromone Collection and Bioassay

The insects were from a laboratory colony maintained at the Northern Grain Insects Laboratory, Brookings, South Dakota, by the method of Jackson (1985). The colony was begun in Brookings, in August 1981, with eggs obtained from a laboratory colony at the USDA, ARS, Vegetable Insects Research Laboratory, Charleston, South Carolina. Virgin females were isolated from males within 24 hr of emergence and held in screened cages (30 × 30 × 30 cm) for three days before being placed in the pheromone collection chamber.

The pheromone collection system and methods were the same as those used to collect volatiles from the southern corn rootworm (SCR), *Diabrotica undecimpunctata howardi* Barber (Guss et al., 1983b). The collection was performed at 24°C with a 12-hr photophase and the pheromone was collected on Porapak Q (Byrne et al., 1975).

During the initial isolation stages, a laboratory bioassay similar to that used for the SCR (Guss et al., 1982) was employed. In this array, four or five virgin male beetles were placed in a Petri dish (150 × 15 mm) and allowed to acclimate for 15 min. A test extract or fraction in 1–5 μl of hexane was applied to a 5-mm^2 piece of filter paper, the solvent was evaporated for about 10 min, and then the paper was placed into the dish. Responses of beetles toward the treated paper, antennal waving, and copulatory behavior were considered evidence of the presence of the pheromone.

Field bioassays were conducted with synthesized putative pheromone and crude and purified natural material from the Porapak Q collectors. Wing traps (Pherocon 1C-type, Trece, Inc., Salinas, California) were baited with various doses of these materials applied (in 200 μl of hexane) to 5.5-cm filter paper circles or to methylene chloride-extracted rubber septa (A.H. Thomas, Philadelphia, Pennsylvania, No. 8753-D22).

The first test was conducted in a field of sweet potato in the vicinity of Homestead, Florida, on November 6, 1985. On the morning of the test, the air temperature was about 18°C at 9:00 AM with winds about 6–10 mph and increasing. The afternoon temperature was about 20°C with winds 10–15 mph.

The traps were baited with filter paper formulations of 1 μg of the purified natural pheromone, a crude extract that contained 1 μg of the natural pheromone, and 1, 4, 10, 100, and 1000 μg of synthetic racemic 6,12-dimethylpentadecan-2-one and a hexane blank. Treatments were assigned randomly to traps within each replicate. Traps were placed 25 m apart in a row across the field perpendicular to the prevailing wind. Two replicates (16 traps in a row) were conducted beginning at 8:45 AM and terminating at 11:30 AM. The experiment was then moved 400 m upwind in the field and two replicates were conducted with rebaited traps in a new random order from 1:40 PM until 9:00 AM the next morning. An observer was assigned to each replicate, and the cumulative total males captured in each trap was recorded at 15-min intervals from 8:45 to 9:30 AM (morning test), from 1:40 to 2:30 PM, at 5:00 PM, and from 8:30 to 9:00 AM the next morning. Tests were conducted during the day because preliminary trapping observations indicated that night temperatures during this period were too cool for BCB flight.

The second test was conducted in a field of mature yellow crook-neck squash in the vicinity of Gainesville, Florida, from November 14 to November 20, 1985. Traps were placed 50 m apart in a row across the field and perpendicular to the prevailing wind. Treatments were 0.03, 0.1, 0.3, 1.0, 3.0, 10, and 30 mg of racemic 6,12-dimethylpentadecan-2-one, and a hexane blank formulated on rubber septa. The baits were not renewed during the test. The traps were checked daily and captured males were removed and counted, at which time the trap and bait were moved to the next trapping location in the line.

Release rates of synthetic pheromone were measured by collecting volatiles released by the septa and analyzing them by gas–liquid chromatography (GLC). Septa were loaded with the same doses used in the field tests and aired at room temperature for 24 hr prior to release-rate measurement. Then a septum was placed in a 15-mm (ID) × 20-mm-long stainless-steel tube, and compressed air, purified by passage through a charcoal filter, was passed over it at 1 liter/min for 1 hr. Volatiles entrained in the airstream were trapped on a small charcoal filter that was subsequently extracted with methylene chloride. After addition of internal standards, the extract was analyzed by capillary GLC.

Pheromone Purification

Volatiles were extracted from Porapak Q filters with 50 ml of ether–hexane (60:40) by agitation for 24 hr. The extract was concentrated with a stream of N_2, and the concentrate was subjected to preparative GLC without further treatment.

Micropreparative GLC was performed with a Varian model 1400 gas chromatograph equipped with a flame ionization detector. The pheromone was purified using two packed columns: 10% OV-101 on 60–80 mesh Chromosorb W (glass column 2 × 2 mm ID) and 7.5% Carbowax 20 M on 60–80 mesh Chromosorb W (glass column 2 m × 2 mm ID). The injection port and column temperatures were both 200°C when operating the OV-101 column and 180°C when operating the Carbowax 20 M column. Detector temperature was 250°C. The carrier gas (He) flow rate through each column was 20 ml/min.

The chromatograph was modified to accommodate a 90:10 effluent splitter and an external, Dry Ice–acetone-cooled fraction collector (Brownlee and Silverstein, 1968). Fractions were collected in 1.5-mm (ID) × 305-mm glass capillary tubes and eluted with about 10 μl of hexane.

Synthesized pheromone was purified by preparative high-performance liquid chromatography (HPLC) using a 4.6-mm (ID) × 25-cm stainless-steel column packed with Adsorbosphere C18 (5 μm) (Alltech/Applied Science, Deerfield, Illinois). The methanol–water (90:10) mobile phase was pumped through the column at 1.0 ml/min, and the eluting components were detected with a Waters model R401 differential refractometer. The 6,12-dimethylpentadecan-2-one eluted from this system in 17.5 min.

Pheromone Analysis and Identification

Natural and synthesized pheromone was analyzed on 50-m × 0.25-mm-ID fused silica capillary columns in a Hewlett-Packard 5790 gas chromatograph equipped with a split–splitless injector system (30-sec split delay) at a carrier gas (He) linear flow rate of 18 cm/sec. The OV-101 column was operated at 60°C for 1 min after injection, temperature programmed at 30°C/min to 200°C, and then operated isothermally. The Carbowax 20 M column was operated at 60°C for 2 min after injection, programmed at 30°C/min to 210°C and then operated isothermally.

Mass spectra were obtained with a Nermag R10-10 mass spectrometer equipped with a chemical ionization (CI)/electron impact (EI) source and interfaced with a Varian Vista model 6000 gas chromatograph equipped with a split–splitless injection system. Samples were introduced into the mass spectrometer source through a 50-m × 0.25-mm-ID fused silica OV-101 column operated at 60°C for 2 min, then programmed at 32°C/min to 230°C and operated isothermally. The linear flow velocity of the carrier gas (He) was 18 cm/

sec. The spectrometer was interfaced with a Digital PDP 11/23 computer for collection and analysis of the data.

Hydrogenoloysis in the inlet of the GC interfaced to the mass spectrometer was performed by the method of Beroza and Sarmiento (1963, 1964). About 6 cm of a glass insert (1 mm ID) was filled with 1% neutral Pd catalyst on Gaschrom W and placed in the injection port ahead of the OV-101 column. The catalyst was maintained at 285°C for the hydrogenolysis, and H_2 was used as the carrier gas at a flow rate of 20 ml/min.

PMR analysis was performed with a Nicolet 300 MHz Fourier transform NMR spectrometer interfaced to a Nicolet model 1280 data system (16K data points, 10 μsec pulse). Samples purified by micropreparative GLC (about 3 μg total pheromone) were transferred from the glass capillary, using benzene-D6, into an NMR tube, the top of which was 5 mm (OD), with a 50 × 2-mm (OD) coaxial extension on the bottom (Wilmad Glass Co., Buena, New Jersey, catalog No. 507 with WGS-5BL stem).

Synthesis

1-Bromo-4-methyl-3-heptene (1). This was reported previously by Guss et al. (1983b), and its preparation has been described in detail by Kulesza et al. (1969). It was prepared in 79% yield; bp 68–70°C at 7 mm (Kuleza et al. reported bp 51–52°C at 2 mm). Its IR and NMR spectra were in complete agreement with its assigned structure.

1-Bromo-4-methylheptane (2). This was prepared from (1) in 70% yield by hydrogenation over platinum oxide in glacial acetic acid (Guss et al., 1983b); bp 67–70°C at 8.5 mm. Its IR and NMR spectra were in agreement with the assigned structure and reported values (Guss et al., 1983b).

8-Methyl-undecan-2-one (3). The lithium reagent was prepared from 9.66 g (0.05 M) of bromide (2), and 0.853 g (0.123 M) of lithium metal in 50 ml of dry ether at −150°C and 100 μl of methyl iodide was used to start the reaction. This lithium reagent was converted into an organocopper reagent and reacted with freshly distilled methyl vinyl ketone in the presence of tri-*n*-butylphosphine by the method of Suzuki et al. (1980). Distillation afforded 80% yield of product; bp 58–60°C at 0.5 mm; CI-MS (CH_4) *m/z*, 185 (M + 1); IR (CCl_4), 2960(s), 2940(s), 2880(s), 2860(s), 1723(s), 1468(m), 1380(m), 1358(m), 1160(m); NMR ($CDCl_3$), 0.84 (6H, m), 1.1–1.4 (11H, m), 1.57 (2H, m), 2.12 (3H, s), 2.41 (2H, t).

4-Oxo-pentane Ketal Triphenylphosphonium Bromide (4). The phosphonium salt was prepared in 60% yield by the method of Sonnet et al. (1986); IR ($CHCl_3$), 2950(s), 1438(s), 1110(s), 1030(m), 690(m), 660(m), NMR ($CDCl_3$), 1.22(3H, s), 1.58 (2H, bds), 1.75(2H, m), 2.05(2H, t), 3.9(4H, s), 7.8(15H, m).

6,12-Dimethyl-5-pentadecen-2-one Ethylene Ketal (5). A hexane solution

of *n*-butyllithium (19.0 ml, 0.038 M) was added dropwise at 0°C to a suspension of 20 g (0.038 M) ketal phosphonium bromide (4) in dry THF and stirred for 30 min. The reaction mixture was brought to room temperature, and 3.6 g (0.02 M) of 8-methylundecan-2-one (3) in ether was added dropwise and the mixture was stirred for 6 hr. The reaction mixture was poured into ice water, and the mixture was extracted with hexane. The hexane solution was washed with water and saturated NaCl and dried over Na_2SO_4. Distillation furnished 870 mg (15% yield) of 6,12-dimethyl-5-pentadecen-2-one ethylene ketal (5); bp 120–130°C at 0.7 mm Hg; CI-MS (CH_4) (*m/z*), 297(M + 1); IR(CCl_4), 2960(s), 2940(s), 2880(s), 1467(m), 1380(m), 1240(m), 1160(m), 1220(m), 1090(m), 1060(m), 950(m), 900(m); NMR ($CDCl_3$), 0.85 (6H, m), 1.1–1.7 (17H, m), 1.68 (3H, s), 2.05 (2H, m), 2.13 (3H, s), 3.95 (4H, bds), 5.10 (1H, m).

6,12-Dimethylpentadecan-2-one Ketal (6). The unsaturated ketal (5) (870 mg, 0.003 M in absolute ethanol) was hydrogenated over 50 mg of palladium on charcoal to yield 832 mg of 6,12-dimethylpentadecan-2-one ketal (6); CI-MS (CH_4) *m/z*, 299(M + 1).

6,12-Dimethylpentadecan-2-one (7). A solution of 832 mg (0.003 M) of ketal (6) in acetone–10% H_2SO_4 was stirred for 3 hr. It was then extracted with hexane and the extract washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated in vacuo to yield 695 mg (98%) of 6,12-dimethylpentadecan-2-one (7); CI-MS (CH_4) *m/z*, 255(M + 1); EI-MS (*m/z*), 43(100), 58(82), 59(38), 71(42), 85(24), 95(13), 97(8), 109(10), 110(15), 123(3), 137(2), 151(1), 152(0.5), 153(0.5), 165(1), 196(3), 211(2), 236(6), 254(M+, 0.9); IR (CCl_4), 2960(s), 2930(s), 2850(s), 1735(s), 1460(m), 1375(m), 1360(m); NMR (C_6D_6), 0.875 (3H, d, J = 8.1 Hz), 0.895 (3H, d, *J* = 8.1 Hz), 0.905 (3H, t, *J* = 6.2 Hz), 1.1–1.4(20H, m), 1.64(3H, s), 1.91(2H, t, *J* = 7.3 Hz).

RESULTS AND DISCUSSION

The hexane–ether extracts of Porapak Q filters from the collection chamber elicited behaviors from BCB males in the laboratory bioassay that indicated the presence of the female sex pheromone. Laboratory assay of the fractions obtained by preparative GLC indicated that the pheromonal activity was confined to a single peak eluting at 11 min on OV-101 under the experimental conditions. Further micropreparative chromatography on the Carbowax 20 M-packed column yielded a compound with a purity of greater than 99.5% by analysis on the OV-101 and Carbowax 20 M capillary columns. The retention indices (Kovats, 1965) of this compound, relative to paraffin hydrocarbons, on the OV-101 and Carbowax 20 M capillary GLC columns were 1787 and 2114, respectively.

The methane CI mass spectrum (Figure 1A) established that the molecular weight of the compound was 254 with diagnostic peaks at *m/e* 253(M − 1),

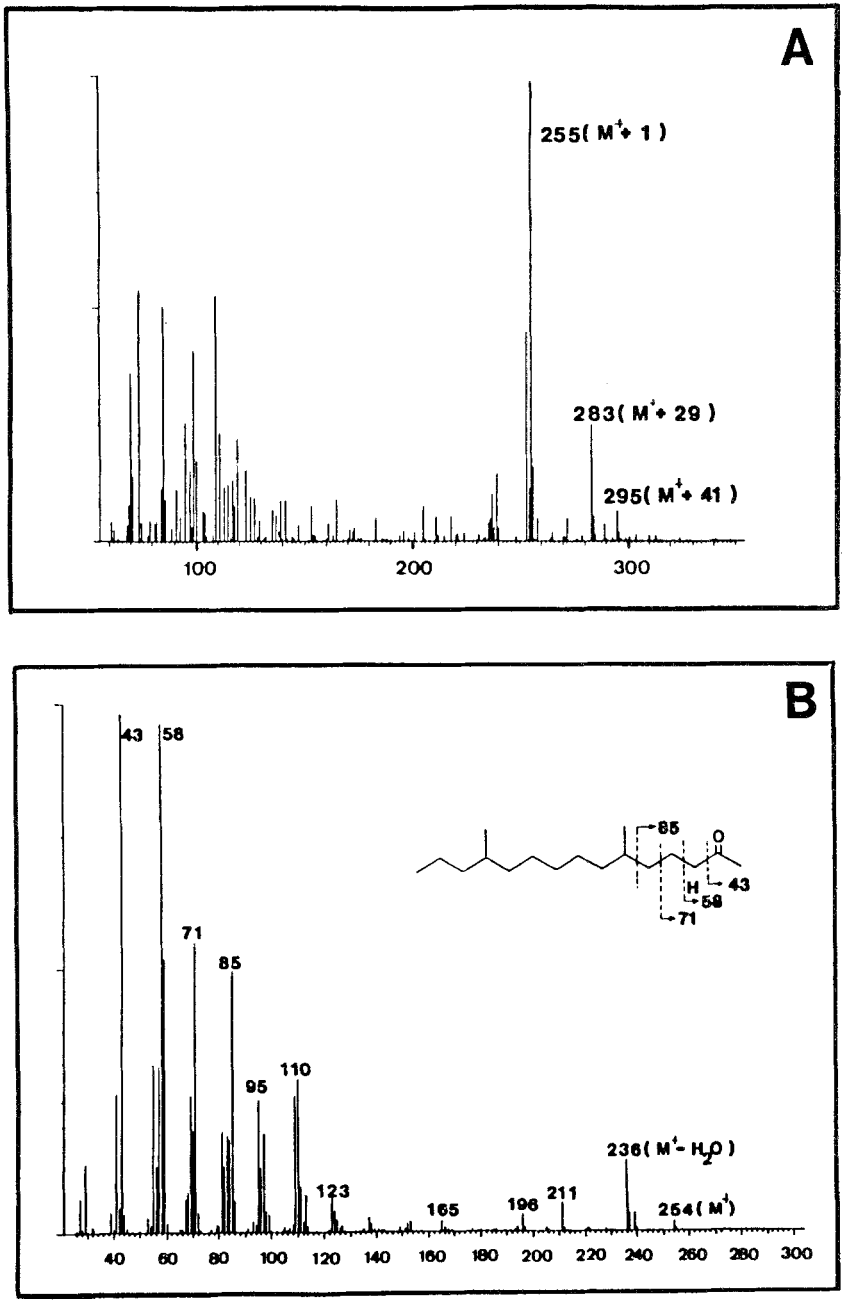


FIG. 1. (A) Chemical ionization (CH_4) and (B) electron impact mass spectra of *D. balteata* pheromone.

255(M + 1), 283(M + 29), and 295(M + 41). In the EI mass spectrum (Figure 1B), the peak at *m/e* 254(M+) confirmed the molecular weight. Furthermore, the EI mass spectrum of this pheromone is very similar to that of the SCR pheromone, 10-methyl-2-tridecanone (Guss et al., 1983b) with a peak at *m/e* 236 indicating the loss of H_2O, and the strong peaks at *m/e* 43 and 58, the weak peak at *m/e* 57, and the moderately strong peak at *m/e* 71 strongly suggesting a methyl ketone with no substitution on the carbons α or β to the carbonyl (Budzikiewicz et al., 1967).

The [^{1}H] NMR spectrum of the purified natural pheromone (Figure 2) supported the above assignment. The signals at δ 1.64 ($\underline{CH_3}CO-$; 3H, s) and 1.92 ($-CO\underline{CH_2}-CH_2-$; 2H, t) confirmed the presence of a 2-oxobutyl moiety in the pheromone molecule. Furthermore, when expanded, the group of peaks from δ 0.86 to 0.91 appeared to consist of three overlapping signals, δ 0.875 (3H, d, J = 8.1 Hz), 0.895 (3H, d, J = 8.1 Hz), and 0.905 (3H, t, J = 6.2 Hz) which suggests the presence of two methyl branches and a terminal methyl in the molecule.

Hydrogenolysis of the pheromone in the GC injector leading to the mass spectrometer source and EI mass spectral analysis of the product yielded the

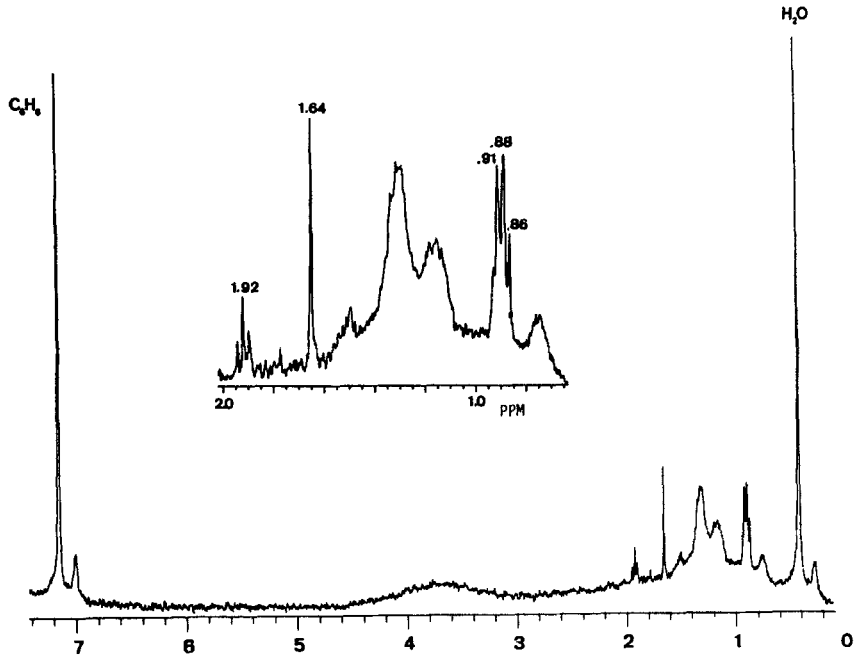


FIG. 2. 300-mHz proton magnetic resonance spectrum of approximately 3 μg of the purified natural *D. balteata* pheromone.

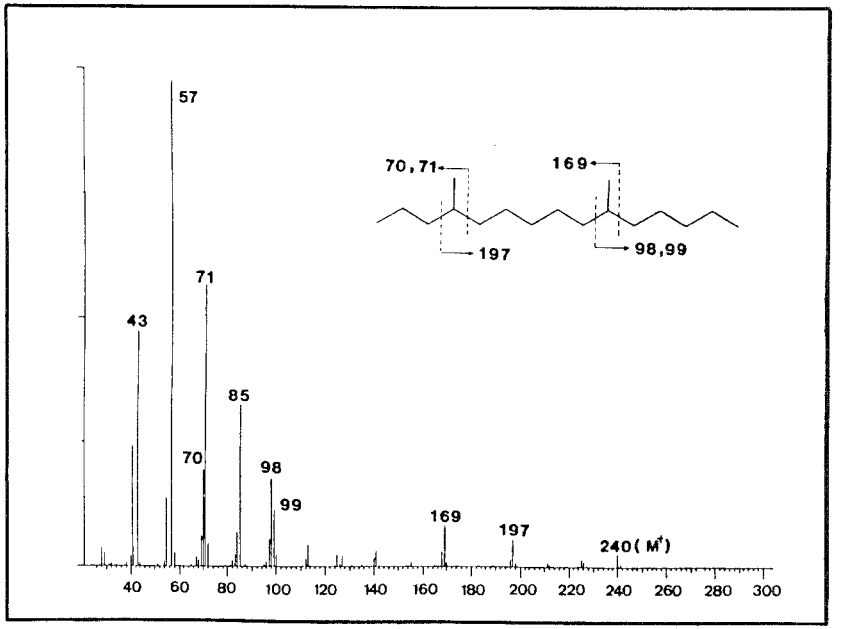
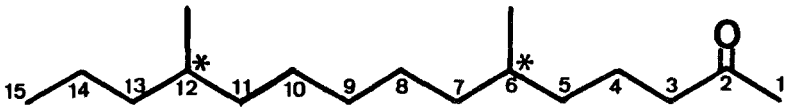


FIG. 3. Electron impact mass spectrum of hydrogenolysis product of *D. balteata* pheromone.

spectrum shown in Figure 3. The peak at 240(M+) confirms the 17-carbon skeleton indicated by mass spectra of the parent compound. The peaks at *m/e* 70, 71, and 197 and at *m/e* 98, 99, and 169 are of greater intensity than would be expected in a normal hydrocarbon and represent preferred cleavage on either side of two methyl branches. These data support a structure with methyl branches on carbons four and ten as indicated in Figure 3. The only other possible structure, with methyl branches on carbons 4 and 6, was ruled out because of the relative intensities of the peaks at *m/e* 98 and 99. The greater intensity of the peak at 98 is consistent with cleavage of the bond between carbons 9 and 10 in 4,10-dimethylpentadecane, with the fragment ion containing only one methyl branch. Cleavage between carbons 6 and 7 of 4,6-dimethylpentadecane yielding a fragment ion containing two methyl branches would be expected to produce peaks at *m/e* 98 and 99, but the peak at 99 should be of greater intensity (Nelson et al., 1972; Nelson and Sukkestad, 1970, 1975; Pomonis et al., 1980; Pomonis and Hakk, 1984). Thus the structure of the hydrogenolysis product was established as 4,10-dimethyl pentadecane. Since the EI mass and PMR spectra of the pheromone indicated that the carbons α and β to the carbonyl must be unsubstituted, the only possible structure is 6,12-dimethylpentadecan-2-one.



Synthesis. The synthesis of racemic 6,12-dimethylpentadecan-2-one (Figure 4) was analogous to that for the sex pheromones of the western corn rootworm (WCR), 8-methyl-2-decanol propanoate, *D. virgifera virgifera* LeConte (Guss et al., 1982), and the SCR (Figure 5) (Guss et al., 1983b). Methylcyclopropyl ketone was allowed to react with *n*-propylmagnesium bromide. The intermediate tertiary carbinol was isomerized to the homoallylic bromide (1) with hydrobromic acid (Julia, 1961). The allic bromide (1) was reduced to 1-bromo-4-methylheptane (2) by hydrogenation with PtO_2. The saturated bromide (2) was converted to the corresponding organocopper reagent which was reacted with methyl vinyl ketone in the presence of tri-*n*-butyl phosphine (Suzuki et al., 1980) to produce 8-methylundecan-2-one (3). A Wittig reaction between the C-12 methylketone (3) and ethylene ketal pentan-2-one phosphonium salt (4), produced a mixture of the *Z* and *E* isomers of the ethylene ketal of 6,12-dimethyl-5-pentadecen-2-one (5) (15% yield). The unsaturated ethylene ketals were hydrogenated and then subjected to acid hydrolysis to produce 6,12-dimethylpentadecan-2-one (7). The final product was purified (>99%) by HPLC and GLC.

The CI and EI mass spectra and the PMR spectrum of pure synthetic racemic 6,12-dimethylpentadecan-2-one were identical with those of the isolated natural pheromone. Additionally the synthetic and natural pheromones cochromatographed, producing only one peak, on each of the two capillary GLC columns.

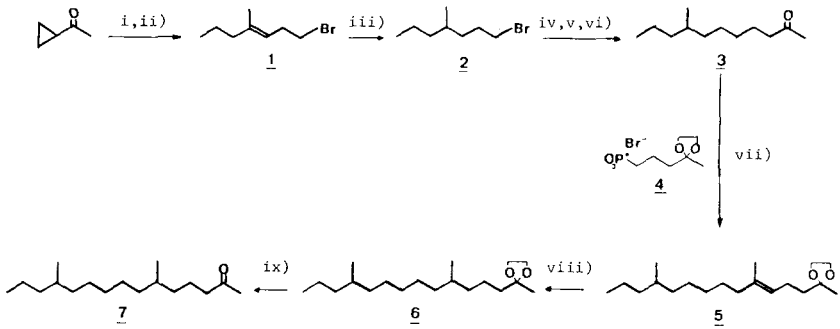


FIG. 4. Synthesis of racemic 6,12-dimethylpentadecan-2-one. (i) C_3H_7MgBr; (ii) HBr; (iii) $H_2/PtO_2/CH_3COOH$; (iv) Li; (v) CuI; (vi) methylvinylketone/$(n\text{-}Bu)_3P$; (vii) *n*-BuLi; (viii) H_2/Pd-C/ETOH; (ix) H_2SO_4.

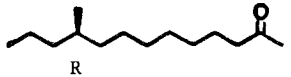
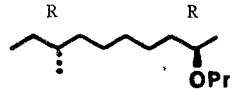
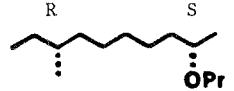
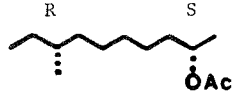
	SPECIES	STEREOCHEMISTRY	STRUCTURES
Fucata species group	D. balteata	unknown	
	D. undecimpunctata howardi (SCR)[1)] D. undecimpunctata undecimpunctata[1)] D. undecimpunctata duodecimnotata[1)]	10R(++) 10R(++) unknown	
Virgifera species group	D. virgifera virgifera (WCR)[2,3)] D. virgifera zeae (MCR)[2,3)] D. barberi (NCR)[2,4)]	2R,8R(++), 2S,8R(+) 2R,8R(++), 2S,8R(+) 2R,8R(++), 2S,8R(--), 2S,8S(-)	
	D. longicornis[2,5)] D. lemniscata[5)] D. porracea[2,3)]	2S,8R(++), 2R,8R(--) 2S,8R(++), 2R,8R(+) 2S,8R(++)	
	D. cristata[6)]	2S,8R(++)	

FIG. 5. Structures and stereochemistry of *Diabrotica* sex pheromones or attractants. (1) Guss et al. (1983b); (2) Guss et al. (1982); (3) Guss et al. (1984); (4) Guss et al. (1985); (5) Krysan et al. (1986); (6) Guss et al. (1983a). Structures shown are the main attractants of the corresponding species. (++), strong attraction; (+), weak attraction; (--), strong inhibition; (−) weak inhibition.

Field Tests. The mean number of males captured in traps baited with filter paper formulations of natural and synthetic materials are summarized in Table 1. Since the purified natural pheromone attracted more males into traps than the crude natural material, the crude material may have included substances that either reduced the release rate or inhibited the response of the males. The captures for 1 μg natural, 4 μg synthetic, and 10 μg synthetic were not statistically different, but were different from all other means. While the number of males captured increased with increasing dose for synthetic pheromone treatments from 1 to 10 μg, linear regression analysis yielded correlation coefficient values slightly less than required for significance at the 5% level of probability (nontransformed data $r = 0.9945$, 1 *df*; the coefficient did not increase with log-transformation). The responses of males to doses > 10 μg was greatly reduced.

The observed captures for the first 2 hr of each replication of the test with filter paper formulations are shown in Table 2. The captures were not dose related for all periods and reveal a complex pattern that is probably related to nonlinear release rates from the filter papers coupled with trap interaction and the depletion of males available to react to each trap. This field was infested heavily with BCB, and reaction to the baits was nearly instantaneous. Males were seen immediately in the vicinity of the 100- and 1000-μg-baited traps but did not enter these traps immediately as they did the other treatments. Again, a dose–response relationship is evident, and regression analysis yields very high correlations for the synthetic treatments 1–10 μg ($r = 0.983$ for the total captures at 2 hr). The increase in captures at the 100-μg level, beginning 30 min after the traps were baited, suggests a decreasing release rate with time.

Apparently the 10-μg dose on filter paper provided a near optimum release

TABLE 1. MEAN CAPTURE OF MALE *Diabrotica balteata* IN TRAPS BAITED WITH NATURAL OR RACEMIC SYNTHETIC 6,12-DIMETHYLPENTADECAN-2-ONE EVAPORATED FROM FILTER PAPERS

Amount (μg)	Treatment	Mean No. males captured per trap, $\bar{X} \pm$ SE ($N = 4$)[a]
0	Hexane blank	0.0 ± 0.0d
1	Natural crude	32.2 ± 7.1b
1	Natural purified	81.7 ± 20.0a
1	Synthetic	65.5 ± 19.1b
4	Synthetic	82.0 ± 18.6a
10	Synthetic	104.2 ± 13.0a
100	Synthetic	37.2 ± 12.4b
1000	Synthetic	8.7 ± 2.9c

[a] Means followed by a common letter are statistically equivalent ($P = 0.05$, data subjected to $\sqrt{x}$ transformation) in Duncan's multiple-range test.

TABLE 2. NUMBER OF MALE *Diabrotica balteata* CAPTURED PER TIME INTERVAL IN TRAPS BAITED WITH FILTER PAPER FORMULATIONS OF NATURAL OR RACEMIC SYNTHETIC 6,12-DIMETHYLPENTADECAN-2-ONE

Treatment						
Natural		Synthetic				
Crude (1 μg)	Pure (1 μg)	1 μg	4 μg	10 μg	100 μg	1000 μg
0–15 min after baiting						
11	71	32	77	86	10	2
15–30 min after baiting						
11	26	23	29	31	1	4
30–45 min after baiting						
4	24	18	15	23	39	6
45 min–2 hr after baiting						
37	145	83	76	138	42	7
Total						
63	266	156	197	278	92	19

rate of the racemic synthetic material. The effect of the stereoisomers cannot be determined clearly at this time, but the similar responses to the 1 μg pure natural and the 4 μg and 10 μg synthetic material suggest that one of the stereoisomers of 6,12-dimethylpentadecan-2-one is a pheromonal constituent.

The response of males to traps baited with rubber septa formulations are summarized in Table 3. Again, a relationship of increasing capture with in-

TABLE 3. MEAN NUMBER OF *Diabrotica balteata* MALES CAPTURED IN TRAPS BAITED WITH RUBBER SEPTA FORMULATIONS OF RACEMIC 6,12-DIMETHYLPENTADECAN-2-ONE

Treatment (μg)	Mean number males captured per trap per replication (±SE)	Release rates from rubber septa (ng/hr)[a]
0	0	
30	5.1 ± 0.5	14
100	13.4 ± 1.9	23
300	24.8 ± 4.3	39
1000	14.5 ± 2.1	124
3000	16.8 ± 1.9	476
10000	9.2 ± 1.1	1322
30000	5.8 ± 1.2	4369

[a]Means of two measurements; flow rate 1 liter/min, ca. 25°C.

creasing dose is evident for traps baited with 30–300 μg of synthetic racemic pheromone ($r = 0.998$ for linear regression of log dose vs. log response). Trap captures declined for treatments above 300 μg. This is the same response pattern observed with the filter paper formulations (Table 1).

The results of the study of release rates of the synthetic pheromone from rubber septa (Table 3) indicate that the release rates continue to increase as the dose is increased over the dose range studied. Thus, the leveling off and decline of trap captures at doses higher than 300 μg/septum cannot be attributed to a leveling off or decrease in the release rate. A similar phenomenon was observed for captures of *D. barberi* Smith and Lawrence, the northern corn rootworm (NCR), in traps baited with racemic 8-methyl-2-decanol propanoate (Figure 5) (Guss et al., 1982). Later this was explained when it was discovered that the NCR is attracted to the 2*R*, 8*R* isomer but that attraction is inhibited by the 2*S*, 8*R* and 2*S*, 8*S* isomers (Guss et al., 1985). On the other hand, the dose–response relationship reported here may be more like that of the WCR, in which rubber septa loaded with 0.25–1000 μg of the 2*R*, 8*R* isomer produced highly correlated captures of males; however, many WCR males ceased their upwind response to the 1-mg bait and attempted to copulate with nearby males (Guss et al., 1984). Thus, BCB males respond positively to one or more of the stereoisomers of 6,12-dimethylpentadecan-2-one and are either inhibited by other stereoisomers of the same compound or, with higher doses, their response to the locus of the active material is diminished. The use of stereoisomers to achieve specificity in their chemical signals is common among the *Diabrotica* studied thus far (Krysan et al., 1986).

The SCR was present in the test areas, and a pheromone of this species, 10-methyl-2-tridecanone, was evaporated from rubber septa baits using doses similar to those formulated in the BCB baits. Neither BCB nor SCR were captured in traps baited with the synthetic racemic pheromone of the other species.

We conclude from the congruence of chemical analytical data and demonstrated field activity in traps with doses equivalent to that of natural material that 6,12-dimethylpentadecan-2-one is a sex pheromone of female BCB. The active enantiomer(s) and the complete role of this chemical in premating communication remain to be determined.

This identification adds to our knowledge of the pheromonal communications systems of the *Diabrotica*: a pattern of chemical structures is beginning to emerge that appears to be related to the taxonomy of this genus. The species of *Diabrotica*, for which the chemical structures of sex attractants are known, fall into two groups (Wilcox, 1972). Those in the *fucata* species group have pheromones characterized by the methyl ketone functionality and a methyl branch on the fourth carbon from the hydrocarbon end of the chain. By contrast, all those taxa is included in the *virgifera* species group are attracted to one or more stereoisomers or analogs of 8-methyl-2-decanol propanoate (Figure 5).

The genus *Diabrotica* includes some 338 species (Wilcox, 1972). It will be interesting to see if these patterns prevail as pheromones from additional species are identified and knowledge accrues on phylogenetic relationships within the genus.

REFERENCES

BEROZA, M., and SARMIENTO, R. 1963. Determination of the carbon skeleton and other structural features of organic compounds by gas chromatography. *Anal. Chem.* 35:1353–1357.

BEROZA, M., and SARMIENTO, R. 1964. Carbon skeleton chromatography using hot-wire thermal conductivity detection. *Anal. Chem.* 36:1744–1750.

BROWNLEE, R.G., and SILVERSTEIN, R.M. 1968. A micropreparative gas chromatograph and a modified carbon skeleton determinator. *Anal. Chem.* 40:2077–2079.

BUDZIKIEWICZ, H., DJERASSI, C., and WILLIAMS, D.H. 1967. Mass Spectrometry of Organic Compounds. Holden-Day, San Francisco, pp. 134–138.

BYRNE, K.J., GORE, W.F., PEARCE, G.T., and SILVERSTEIN, R.M. 1975. Porapak Q collection of airborne organic compounds serving as models for insect pheromones. *J. Chem. Ecol.* 1:1–7.

CUTHBERT, F.P., JR., and JONES, A. 1972. Resistance in sweet potatoes to Coleoptera increased by recurrent selection. *J. Econ. Entomol.* 65:1655–1658.

CUTHBERT, F.P., JR., and REID, W.J. 1964. Studies of sex attractants of banded cucumber beetle. *J. Econ. Entomol.* 57: 247–250.

GUSS, P.L., TUMLINSON, J.H., SONNET, P.E., and PROVEAUX, A.T. 1982. Identification of a female-produced sex pheromone of the western corn rootworm. *J. Chem. Ecol.* 8:545–556.

GUSS, P.L., CARNEY, R.L., SONNET, P.E., and TUMLINSON, J.H. 1983a. Stereospecific sex attractant for *Diabrotica cristata* (Harris) (Coleoptera: Chrysomelidae). *Environ. Entomol.* 12:1296–1297.

GUSS, P.L., TUMLINSON, J.H. SONNET, P.E., and MCLAUGHLIN, J.R. 1983b. Identification of a female-produced sex pheromone from the southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber. *J. Chem. Ecol.* 9:1363–1375.

GUSS, P.L., SONNET, P.E., CARNEY, R.L., BRANSON, T.F., and TUMLINSON, J.H. 1984. Response of *Diabrotica virgifera virgifera, D. V. zeae*, and *D. porracea* to stereoisomers of 8-methyl-2-decyl propanoate. *J. Chem. Ecol.* 10:1123–1131.

GUSS, P.L., SONNET, P.E., CARNEY, R.L., TUMLINSON, J.H., and WILKIN, P.J. 1985. Response of northern corn rootworm, *Diabrotica barberi* Smith and Lawrence, to stereoisomers of 8-methyl-2-decyl propanoate. *J. Chem. Ecol.* 11:21–26.

JACKSON, J.J. 1985. *Diabrotica*, pp. 237–254, *in* P. Singh and R. F. Moore (eds.). Handbook of Insect Rearing. Elsevier, Amsterdam.

JULIA, M. 1961. Nouveaux alcools a chaine isoprenique et leur preparation. Fr. Patent 1,213,486, July 31, 4 pp.

KOVATS, E. 1965. A retention index system. *Adv. Chromatogr.* 1:229–235.

KRYSAN, J.L. 1986. Introduction: biology, distribution and identification of pest *Diabrotica*, pp. 1–23, *in* J.L. Krysan and T.A. Miller (eds.). Methods for the Study of Pest Diabrotica. Springer-Verlag, New York.

KRYSAN, J.L., WILKIN, P.H., TUMLINSON, J.H., SONNET, P.E., CARNEY, R.L., and GUSS, P.L. 1986. Responses of *Diabrotica lemniscata* and *D. longicornis* (Coleoptera: Chrysomelidae) to stereoisomers of 8-methyl-2-decyl propanoate and studies on the pheromone of *D. longicornis*. *Ann. Entomol. Soc. Am.* 79:742–746.

KULESZA, J., GORA, J., and KOSESKA, K. 1969. Synthesis of β-elgenol and its analogs. *Riechst. Aromen Koerperpflegem.* 19:192–200.

MELHUS, I.E., PAINTER, R.H., and SMITH, F.O. 1954. A search for resistance to the injury caused by species of *Diabrotica* in the corns of Guatemala. *Iowa State J. Sci.* 29:75–94.

NELSON, D.R., and SUKKESTAD, D.R. 1970. Normal and branched aliphatic hydrocarbons from the eggs of the tobacco hornworm. *Biochemistry* 9:4601–4611.

NELSON, D.R., and SUKKESTAD, D.R. 1975. Normal and branched alkanes from cast skins of the grasshopper, *Schistocerca vaga* (Scudder). *J. Lipid Res.* 16:12–18.

NELSON, D.R., SUKKESTAD, D.R., and ZAYLSKIE, R.G. 1972. Mass spectra of methyl-branched hydrocarbons from eggs of the tobacco hornworm. *J. Lipid Res.* 13:413–421.

OVERMAN, J.L., and MACCARTER, L.E. 1972. Evaluating seedling of cantaloupe for varietal non-preference-type resistance to *Diabrotica* spp. *J. Econ. Entomol.* 65:1140–1144.

PITRE, H.N., and KANTACK, E.J. 1962. Biology of the banded cucumber beetle, *Diabrotica balteata* in Louisiana. *J. Econ. Entolmol.* 55:904–906.

POMONIS, J.G., and HAKK, H. 1984. Alkanes from surface lipids of sunflower stem weevil, *Cylindrocopturus adspersus* LeConte. *J. Chem. Ecol.* 10:1335–1347.

POMONIS, J.G., NELSON, D.R., and FATLAND, C.L. 1980. Insect hydrocarbons 2. Mass spectra of dimethylalkanes and the effect of the number of methylene units between methyl groups on fragmentation. *J. Chem. Ecol.* 6:965–972.

RISCH, S. 1976. Effect of variety of cowpea (*Vigna unguiculata* L.) on feeding preferences of three chrysomelid beetles, *Certoma ruficornis rogersi*, *Diabrotica balteata* and *Diabrotica adelpha*. *Terrialba* 26:327–330.

SABA, F. 1970. Host plant spectrum and temperature limitations of *Diabrotica balteata*. *Can. Entomol.* 102:684–691.

SCHWARZ, M., JACOBSON, J., and CUTHBERT, F.P., JR. 1971. Chemical studies of the sex attractant of the banded cucumber beetle. *J. Econ. Entomol.* 64:769–770.

SONNET, P.E., PROVEAUX, A.T., ADAMEK, E., SUGIE, H., SATO, R., and TAMAKI, Y. 1987. Stereoisomers and analogs of 14-methyl-1-octadecene, sex pheromone of the peach leafminer moth, *Lyonetra clerkella* L. *J. Chem. Ecol.* 13:547–555.

SUZUKI, M., SUZUKI, T., KAMAGISHI, T., and NOYORI, R. 1980. Organocopper conjugate addition revisited. *Tetrahedron Lett.* 21:1247–1250.

TENG, H.J., WADDILL, V., SLANSKY, F., and STRAYER, J. 1984. Performance and host preference of adult banded cucumber beetles, *Diabrotica balteata*, when offered several crops. *J. Agric. Entomol.* 1:327–330.

WILCOX, J.A. 1972. *Coleopterorum Catalogus Supplementa*. (Ed. 2). *Galerucinae luperini: Pars 78, Fisc.* 2:296–431.

YOUNG, W.R. 1961. Banded cucumber beetle—biological and ecological studies. The Rockefeller Foundation Annual Report 1959–1960.